physiologically inactive histamine-formaldehyde complex was postulated.[2] The following observation is an addition to the above list of inactivation methods.

It was found, that if to histamine solutions of known concentration are added 2 mols. of diazotized sulfanilic acid in the presence of Na_2CO_3 the resulting dye (Pauly's reaction) will not contract the surviving intestinal strip of the guinea pig if the solution is brought to a physiological pH. On the other hand an insufficient molar quantity of the diazonium compound will cause only a partial inactivation of the base. This observation was found to be useful when the presence of histamine is suspected in biological extracts, etc. Because of the possibility that the tyramine-diazotized sulfanilic acid condensation product would also result in an inactivation of the tyramine or an analogous effect might be observed if smooth muscle contractive protein split products were treated as above, this test should be taken only as additional evidence for the presence or absence of histamine.

6230

Gelatin as an Opsonizing Substance.

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Since the introduction of the term opsonin by Wright and Douglas the conception of the mechanism of action of opsonins has rather constantly tended to point toward a deposition of such substances on the surface of the particle to be phagocytized. Recent studies are well exemplified by the experiments of Mudd, Lucké, McCutcheon and Strumia,[1] who have shown that the globulin fraction of immune sera contains a substance or substances which will unite with the antigen, bacterial cells or inert colloidal particles coated with a protein, in such a fashion that surface properties of the antigen as cohesiveness and cataphoresis are altered in association with such immunological reactions as agglutination and increased phagocytosis.

[2] Kendall, A. I., *J. Infect. Dis.*, 1927, **40**, 689; see also ref. 1.

[1] Mudd, Lucké, McCutcheon and Strumia, *J. Exp. Med.*, 1929, **49**, 779; 1930, **52**, 313.

If phagocytosis is stimulated by such a coating on the surface of the antigen, as Mudd and his coworkers postulate, it might be possible to use substances other than serum or its various protein fractions to so prepare the surface of the antigen for phagocytosis. Since the completion of our work Freund[2] has published his experimental studies in which he used tannin and observed phagocytosis of red blood cells in the absence of serum. He was thus able to repeat the earlier experiments of Reiner and Koppz.[3]

Since gelatin has long been used to coat particles of one sort or another with a view of altering their surface physics we began our experiments using this substance. We employed a commercial bacteriological sheet gelatin made up in saline to the designated concentration and adjusted to pH 7.4. The results were also confirmed with a second gelatin furnished by Dr. Gebauer-Fuelnegg and prepared from the skin of hogs by mild chemical extraction.

In our first experiments we made use of the Wright capillary pipette method estimating the percent of polymorphonuclear cells showing phagocytosis, after 15 minutes incubation at 37°C. This method was soon abandoned because it was noted that the blood cells in the presence of gelatin tended to settle out on standing. A method was made use of in which the reacting substances were pipetted into small paraffined tubes which were subjected to gentle mechanical agitation in a water bath for 15 minutes after which smears were made and examined in the usual fashion. Our first experiments were done with a suspension of living staphylococci but we later used a killed suspension of *Streptococcus scarlatinae* as described by Jung and Day.[4]

TABLE I.
Varying Concentrations of Normal Serum and Gelatin as Opsonizing Agents.

% Concentration of Serum	100	50	25	12.5	6.2		
% Phagocytosis	35	24	17	16	16		
% Concentration of Gelatin	10	5	4	3	2	1	0.5
% Phagocytosis	36	30	25	26	32	19	8

Saline control—4% Phagocytosis.

The table shows the effect of varying concentrations of gelatin or human serum on the phagocytosis of the killed streptococcus suspension. In experiments of this type 0.05 cc. of the opsonin (serum or gelatin dilution) or saline in the control, 0.05 cc. of bacterial suspension and 0.10 cc. of washed human blood cells were the

[2] Freund, J., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 876.

[3] Reiner and Koppz, *Z. f. Immunitäts.*, 1929, **61**, 397.

[4] Jung and Day, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1080.

quantities employed. These results indicate that gelatin can serve as an opsonin increasing the phagocytosis from 4% in the saline control to 36% in the case of 10% gelatin. Although in this particular experiment the gelatin curve happens to indicate a slightly greater phagocytosis with the concentrations of gelatin used than with the serum, in many of our experiments, when undiluted serum and gelatin in 2% or 3% concentrations were compared, the phagocytosis in the serum usually ran from 30% to 40% while the gelatin results ranged from 20% to 30%. The saline controls rarely showed more than 5% phagocytosis.

We have tried a limited number of other substances with a view of explaining the mechanism of the opsonic action of gelatin.

Fresh white of egg in concentrations of 12% or less has failed to give any evidence of aiding phagocytosis in the absence of serum. Even in concentrations of 25% to 50% the egg white yielded negative results except on one occasion when 16% phagocytosis was observed with a 1-1 dilution. Since the relative viscosity of a 25% egg white solution is greater than that of a 2% gelatin solution, viscosity may not be the important factor in explaining the action.

The buffer values of nutrient broth, 1% gelatin and saline, were compared and found to decrease in that order. Since phagocytosis takes place in the presence of 1% gelatin and is absent in the presence of the more highly buffered nutrient broth, it is possible that the buffering value of the gelatin is not sufficient to explain its action as an opsonic substance.

Attempts have been made to wash bacterial cells sensitized with gelatin but the results indicate that the gelatin is easily removed by washing. Hektoen[5] has shown that in immune serum as well as in normal serum the heat stabile opsonic substance is firmly held to the bacterial or blood cell surface despite washing and, although alone has little effect on phagocytosis, with a small amount of normal serum greatly increases phagocytosis. We were unable to demonstrate that gelatin possessed any analogous property.

Conclusion. We have shown that washed human polymorphonuclear leukocytes will phagocytize staphylococci or streptococci in the presence of gelatin without the addition of serum.

[5] Hektoen, *J. Inf. Dis.*, 1909, **6**, 66.